Fusicatenibacter saccharivorans gen. nov., sp. nov., isolated from human faeces

Toshihiko Takada, Takashi Kurakawa, Hirokazu Tsuji and Koji Nomoto

Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi, Tokyo, 186-8650, Japan

Three Gram-stain-positive, obligately anaerobic, non-motile, non-spore-forming, spindle-shaped bacterial strains (HT03-11\textsuperscript{T}, KO-38 and TT-111), isolated from human faeces were characterized by phenotypic and molecular taxonomic methods. Comparative 16S rRNA gene sequencing showed that the strains were highly related to each other genetically (displaying $>99\%$ sequence similarity) and represented a previously unknown subsline within the Blautia coccoides rRNA group of organisms (cluster XIVa). The closest phylogenetic neighbours of strain HT03-11\textsuperscript{T} were Clostridium bolteae WAL 16351\textsuperscript{T} (93.7\% 16S rRNA gene sequence similarity) and Clostridium saccharolyticum WM1\textsuperscript{T} (93.7\% similarity). All isolates produced lactic acid, formic acid, acetic acid and succinic acid as fermentation end products from glucose. Their chemotaxonomic properties included lysine as the cell wall diamino acid and $C_{16:0}$, $C_{18:1}\Delta7c$ DMA and $C_{16:0}$ DMA as the major fatty acids. The G+C contents of the genomic DNA were 46.9–47.2 mol\% (HPLC). Several phenotypic and chemotaxonomic characteristics could be readily used to differentiate the isolates from phylogenetically related clostridia. Therefore, strains HT03-11\textsuperscript{T}, KO-38 and TT-111 represent a novel species in a new genus of the family Lachnospiraceae, for which the name Fusicatenibacter saccharivorans gen. nov., sp. nov. is proposed. The type strain of the type species is HT03-11\textsuperscript{T} (=YIT 12554\textsuperscript{T}=JCM 18507\textsuperscript{T}=DSM 26062\textsuperscript{T}).

The diversity of bacterial flora in the human large intestine has been revealed through detailed analyses using molecular biological approaches (Harmsen et al., 2002; Eckburg et al., 2005; van den Bogert et al., 2011). In recent years, metagenomics-based methods have revealed the bacterial composition and function in the human gut microbial communities (Arumugam et al., 2011, Human Microbiome Project Consortium, 2012). In the large intestine of a healthy person, the flora consists mainly of the Bacteroides group, Blautia coccoides group (cluster XIVa), Clostridium leptum subgroup (cluster IV), Bifidobacterium, Atopobium cluster and Eubacterium cylindroides group, accounting for approximately 90\% of all colonic bacteria (Harmsen et al., 2002). These bacteria constitute a balanced flora in the intestine and perform beneficial functions for the host, such as inhibition of the growth of pathogenic bacteria, decomposition of food, synthesis of vitamins and immune system activation (Gibson & Roberfroid., 1995; Prakash et al., 2011).

The Blautia coccoides group is the dominant bacterial group in the human intestine; approximately $10^8$–$10^{10}$ bacteria of this group are contained in 1 g of faeces (wet weight) from a healthy person, accounting for approximately 20–30\% of all bacteria (Harmsen et al., 2002; Matsuï et al., 2004). This group has been shown to consist of a variety of bacterial species, including a number of unidentified bacteria not belonging to any of the known species (Hayashi et al., 2006; Maukonen et al., 2006). Functional studies of the Blautia coccoides group identified bacterial species that produce butyrate, a short-chain fatty acid that serves as a source of energy for intestinal epithelial cells and has beneficial health effects for the host, within the species of the genera Eubacterium, Roseburia and Coprococcus of the Blautia coccoides group (Aminov et al., 2006; Louis & Flint, 2009). Subsequent genetic analyses revealed the involvement of butyryl-CoA:acetetyl-CoA-transformer activity in butyrate synthesis (Louis et al., 2004) while mouse studies revealed that Bacteroides thetaiotaomicron produces acetate through assimilation of glucans and Eubacterium rectale converts the acetate to butyrate. Furthermore, some studies in humans have shown a positive correlation between carbohydrate intake and butyrate production and the number of butyrate-producing bacteria of the Blautia coccoides group (Duncan et al., 2007), suggesting a major role for the Blautia coccoides group in butyrate metabolism in the human intestine.

While a number of bacteria in the Blautia coccoides group have been identified as beneficial to human health, the biology and functions of unidentified bacterial species have not been fully characterized. In this study, we recovered strains of a hitherto unknown obligately anaerobic,
Gram-stain-positive, spindle-shaped bacterium from human faecal samples using traditional culturing methodologies, and characterized the strains by phenotypic and molecular taxonomic methods.

Strains HT03-11T, KO-38 and TT-111 were isolated from three healthy human adults, subjects HT, KO and TT, 44, 26 and 46 years old, respectively, none of whom had undergone antibiotic therapy in the previous 6 months. For isolation, fresh faecal samples were collected and immediately transferred anaerobically. The faecal samples were homogenized and subsequently diluted with pre-reduced PBS solution without a metal salt [PBS(−); Nissui Pharmaceutical] and spread onto Brucella agar (Difco) supplemented with 5 % sheep blood. Plates were incubated at 37 °C for 3 days in an anaerobic glove box (Coy Laboratory Products) which contained 88 % nitrogen, 7 % hydrogen and 5 % carbon dioxide. The colonies were repeatedly picked and streaked until a pure culture was obtained. For morphological and physiological studies, the strain was grown on modified Gifu anaerobic medium (GAM) (Nissui Pharmaceutical) agar.

The bacterial cells were mechanically disrupted with glass beads (diameter, 0.1 mm) and the ShakeMaster (BioMedical Science). The DNA in the disrupted bacterial cell was purified using the DNeasy Blood & Tissue kit (Qiagen) according to the protocol provided by the manufacturer. For phylogenetic analysis, the 16S rRNA gene of the strain was amplified with 8F and 15R bacterial universal primers (Irisawa & Okada, 2009). PCR was performed with an rTaq DNA polymerase kit (TaKaRa). Each 30 µl reaction mixture had a concentration of 0.2 mM dNTPs, 1.5 mM MgCl₂, 1.5 U rTaq polymerase, primers 8F and 15R (1 µM each) and 1 µl DNA solution. The PCR amplification was carried out with initial denaturation at 95 °C, for 5 min and followed by 35 cycles of denaturation (95 °C, 30 s), annealing (55 °C, 30 s) and extension (72 °C, 1 min). A final extension step was performed at 72 °C for 5 min. The PCR product was purified using a High Pure PCR Product Purification kit (Roche Diagnostics) as per the manufacturer’s instructions. The 16S rRNA gene nucleotide sequences were determined by cycle sequencing using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer’s instructions. Sequences were read with an automatic sequence analyser (ABI PRISM 3130 DNA sequencer). Closely related sequences were retrieved from the DNA DataBank of Japan (DDJB, http://www.ddbj.nig.ac.jp) using the FASTA program (Lipman & Pearson, 1985). Sequences were aligned and used to produce an unrooted phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987) using CLUSTAL W (version 2.1) (Thompson et al., 1994). The stability of the groupings was estimated by bootstrap analysis (1000 replications) in CLUSTAL W. The tree was visualized using the TreeView program (version 1.6.6) (Page, 1996).

Almost-complete 16S rRNA gene sequences of strains HT03-11T (1423 bp), KO-38 (1392 bp) and TT-111 (1508 bp) were determined. Pairwise analysis showed the three isolates to all be phylogenetically closely related to each other, with isolates displaying >99 % sequence similarity. Sequence searches of the Ribosomal Database Project (http://rdp.cme.msu.edu) library for species with validly published names revealed that the unknown organisms were members of the Clostridium subphylum of Gram-stain-positive bacteria and were most closely related to species of the Blautia cocoides rRNA group [cluster XIVa, (Collins et al., 1994)]. A tree reconstructed by the neighbour-joining method depicting the phylogenetic affinity of the unknown bacterium as exemplified by strains HT03-11T, KO-38 and TT-111 is shown in Fig. 1 and confirmed the placement of the unidentified bacterium in the Blautia cocoides rRNA group. The database searches also revealed that many uncultured bacterial clones (Roger et al., 2010; Gill et al., 2006; Eckburg et al., 2005; Mai et al., 2006; Dethlefsen et al., 2008) were closely related (>98 %) to strain HT03-11T, KO-38 or TT-111, indicating that these strains are present in human faeces as common members of the human indigenous microbiota. The closest phylogenetic neighbours of strain HT03-11T were Clostridium bolteae WAL 16351T (93.7 % 16S rRNA gene sequence similarity) and Clostridium saccharolyticum WM1T (93.7 % similarity). It is evident from the results of the taxonomic study that the Gram-stain-positive strains HT03-11T, KO-38 and TT-111 recovered from human faeces represent hitherto unknown species.

The Gram reaction was determined using Gram staining. Cellular morphology was observed for cells grown in pre-reduced anaerobically sterilized GAM broth supplemented with vitamin K₁ and haemin at 37 °C for 20 h using phase-contrast microscopy (BX-50; Olympus) and scanning electron microscopy (S3400N; Hitachi). For sample preparation for SEM, cells were placed on Sempore (JEOL Datum), fixed with glutaraldehyde and OsO₄, critical point dried and coated with osmium plasma. Fig. S1 (available in IJSEM Online), depicts a scanning electron micrograph of strain HT03-11T. For spore formation, cells were cultured at 37 °C for 7 days using Duncan & Strong (DS) medium (Duncan & Strong, 1968).

The end products of bacterial metabolism, after cultivation in the GAM broth for 3 days at 37 °C, were analysed by HPLC according to a previously reported procedure (Chonan et al., 1995). Biochemical characteristics were determined using the API 20A and API ZYM systems (API bioMérieux) according to the manufacturer’s instructions. Pre-reduced peptone-yeast extract (PY) (Holdeman et al., 1977) was used as the basal medium for API20A. For additional examinations, inulin was added at a 1 % concentration to the PY medium. The culture was incubated anaerobically at 37 °C for 3 days and bacterial growth was monitored by examining the change in pH. The growth response results were classified as follows: positive response, <pH 5.5; weak response, pH 5.6–6.0 on PY medium (initial pH; 7.0); and negative response, no pH change. Each assay was performed in triplicate. Hydrogen sulfide production, indole formation and motility were tested using hydrogen sulfide indole motility (SIM) medium, according to the protocol of
Cells for chemotaxonomic analyses were harvested from Brucella broth (BD) supplemented with 1% glucose after anaerobic cultivation for 20 h at 37 °C. The composition of cellular fatty acids was analysed by using the Sherlock Microbial Identification (MIDI) System version 6.0 according to the manufacturer’s instructions. Polar lipids were extracted according to the method described by Tindall et al. (1990). The cell wall peptidoglycan was isolated after disruption of the cells by sonication with glass beads and subsequent total hydrolysis (4 M HCl, 100 °C, 16 h) (Komagata & Suzuki, 1987). The amino acid isomers in cell wall hydrolysates were examined by the method described by Nozawa et al. (2007) using a liquid chromatograph–mass spectrometer (QSTAR Elite Hybrid Quadrupole TOF; AB SCIEX). The molar ratios of amino acids were determined by HPLC (Alliance 2695 HPLC system; Waters) equipped with a fluorescence detector (model 2475 Fluorescence Detector; Waters) and an AccQ-Tag column (3.9 × 150 mm; Waters) and using an AccQ-Fluor reagent KIT (6-aminoquinolyl-N-hydroxysuccinimidyl carbonate; Waters) for derivatization.

Cells of HT03-11ᵀ, KO-38 and TT-111 were Gram-stain-positive, obligately anaerobic, non-motile, non-spore-forming. The cell wall peptidoglycan was isolated after disruption of the cells by sonication with glass beads and subsequent total hydrolysis (4 M HCl, 100 °C, 16 h) (Komagata & Suzuki, 1987). The amino acid isomers in cell wall hydrolysates were examined by the method described by Nozawa et al. (2007) using a liquid chromatograph–mass spectrometer (QSTAR Elite Hybrid Quadrupole TOF; AB SCIEX). The molar ratios of amino acids were determined by HPLC (Alliance 2695 HPLC system; Waters) equipped with a fluorescence detector (model 2475 Fluorescence Detector; Waters) and an AccQ-Tag column (3.9 × 150 mm; Waters) and using an AccQ-Fluor reagent KIT (6-aminoquinolyl-N-hydroxysuccinimidyl carbonate; Waters) for derivatization.

Fig. 1. Neighbour-joining phylogenetic tree, based on a comparison of partial (1300 nt) 16S rRNA gene sequences, showing the relationships of Fusicatenibacter saccharivorans gen. nov., sp. nov. and related taxa within the Blautia coccoides rRNA XIVa cluster. Clostridium propionicum JCM 1430ᵀ (cluster IV of the genus Clostridium) was used as the outgroup. Bootstrap percentages (based on 1000 replications) are shown at branching points. DDBJ accession numbers for each 16S rRNA gene sequence are given in parentheses. Bar, 0.02 substitutions per site.
rods. Cells were approximately 0.5–1.0 μm in width and 1.5–15.0 μm in length, spindle-shaped and arranged in pairs and short chains (Fig. S1). Colonies after 2 days at 37 °C of anaerobic incubation on Brucella blood agar plates were 2–3 mm in diameter, non-haemolytic, flat, brownish and irregular. Detailed biochemical characteristics of strains HT03-11T, KO-38 and TT-111 are given in the species description and in Table 1.

Purified cell walls of strains HT03-11T, KO-38 and TT-111 contained lysine, glutamate and alanine in the molar ratio 0.7 : 1.0 : 1.1. Phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), two aminoglycolipids (AGL1 and AGL2), a phospholipid (PL3) and an unidentified lipid (L2) were present in strains HT03-11T, KO-38 and TT-111 as the common polar lipids (Fig. S2).

It is evident from Table 1 that several phenotypic properties readily distinguished strains HT03-11T, KO-38 and TT-111 from other phylogenetically related species, namely C. bolteae WAL 16351T and C. saccharolyticum WM 1T. C. bolteae WAL 16351T (Song et al., 2003) did not produce acid from D-mannose, salicin or inulin and had no enzyme activity of esterase (C4), esterase lipase (C8), α-galactosidase, β-galactosidase and α-glucosidase. Also, C. saccharolyticum WM 1T (Murray et al., 1982) did not produce acid either from D-sorbitol or inulin and had no enzyme activity of esterase lipase (C8) and α-galactosidase. In addition, the DNA G+C content of strains HT03-11T, KO-38 and TT-111 (46.9–47.2 mol%) were far higher than that of C. saccharolyticum WM 1T (28.0 mol%). Furthermore, the major fatty acids of strains HT03-11T, KO-38 and TT-111 were C16:0 (23.8–25.9 %), C18:1ω7c DMA (19.3–28.5 %) and C16:0 DMA (8.7–14.2 %); however, fatty acid C16:0 was present in C. bolteae YIT 12645T in minor amounts (5.2 %) and C16:1ω7c DMA, C14:0 and C16:1ω7c were present in C. saccharolyticum YIT 12747T in major amounts (14.5 %, 13.4 % and 11.5 %, respectively; Table S1).

Therefore, based on the physiological, biochemical and chemotaxonomic characteristics, strains HT03-11T, KO-38 and TT-111 represent a novel species in a new genus of the family Lachnospiraceae, for which the name Fusicatenibacter saccharivorans gen. nov., sp. nov. is proposed.

### Description of Fusicatenibacter gen. nov.

**Fusicatenibacter** (fu.si.ca.te.ni.bac.‘ter. L. n. *fusus* a spindle; L. n. *catena* chain; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Fusicatenibacter* a spindle-shaped chain rod).

Anaerobic, Gram-stain-positive, non-spore-forming rods, which are approximately 0.5–1.0 μm wide and 1.5–15.0 μm long, spindle-shaped and arranged in pairs and short chains. The major polar lipid profile consists of PC, PG and two aminoglycolipids. Lysine as the diamino acid is contained in the cell-wall peptidoglycan. Major cellular fatty acids are C16:0, C18:1ω7c DMA and C16:0 DMA. The type, and currently only, species of the genus is *Fusicatenibacter saccharivorans*.

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**Table 1.** Major characteristics of strains HT03-11T, KO-38 and TT-111 and the type strains of closely related members of the *Blaufia coccosoides* rRNA XIVA cluster

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>α-Glucosidase</td>
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<td>L, F, A, s</td>
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<td>47.2</td>
<td>47.2</td>
<td>50.5</td>
<td>28.0</td>
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</table>

*A*, acetic acid; *F*, formate; *L*, lactate; *S* or *s*, succinic acid. Minor end products are indicated by lower-case letters.

†m-Dpm, meso-diaminopimelic acid.
Description of *Fusicatenibacter saccharivorans* sp. nov.

*Fusicatenibacter saccharivorans* (sac.cha.ri.vo’rans. L. n. saccharum sugar; L. part. adj. vorans devouring, digesting; N.L. part. adj. saccharivorans sugar-digesting).

Exhibits the following properties in addition to those given in the genus description. On Brucella blood agar, non-haemolytic, flat, brownish and irregular colonies with a diameter of 2–3 mm form after 48 h at 37 °C. Positive result in tests for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, α- galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, naphthol-AS-BI-phosphohydrolase (weak reaction) and β-glucuronidase (weak reaction) activities, but negative results for lipase (C14), valine arylamidase, cysteine arylamidase, trypsin and α-chymotrypsin activities. Ammonia, indole and hydrogen sulfide are not produced and urease is also absent. Aesculin is hydrolysed but gelatin is not. Produces acid from glucose, lactose, sucrose, maltose, salicin, xylose, arabinose, cellobiose, mannose, raffinose, sorbitol, rhamnose, trehalose, inulin and melizitose (weak reaction), but not from mannitol or glycerol. Forms formate, lactate, acetate and succinate as major products from glucose fermentation.

The type strain is HT03-11T (YIT 12554T = JCM 18507T = DSM 26062T). The DNA G+C content of the type strain is 46.9 mol%. Strains KO-38 and TT-111 are additional strains of the species.

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References


